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N9-Substituted 2,4-Diaminoquinazolines: Synthesis and Biological Evaluation of Lipophilic Inhibitors of *Pneumocystis carinii* and *Toxoplasma gondii* Dihydrofolate Reductase

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Abstract

N9-substituted 2,4-diaminoquinazolines were synthesized and evaluated as inhibitors of *Pneumocystis carinii* (pc) and *Toxoplasma gondii* (tg) dihydrofolate reductase (DHFR). Reduction of commercially available 2,4-diamino-6-nitroquinazoline **14** with Raney nickel afforded 2,4,6-triaminoquinazoline **15**. Reductive amination of **15** with the appropriate benzaldehydes or naphthaldehydes, followed by N9-alkylation, afforded the target compounds **5–13**. In the 2,5-dimethoxybenzylamino substituted quinazoline analogues, replacement of the N9—CH₃ group of **4** with the N9—C₂H₅ group of **8** resulted in a 9- and 8-fold increase in potency against pcDHFR and tgDHFR, respectively. The N9—C₂H₅ substituted compound **8** was highly potent, with IC₅₀ values of 9.9 and 3.7 nM against pcDHFR and tgDHFR, respectively. N9-propyl and N9-cyclopropyl methyl substitutions did not afford further increases in potency. This study indicates that the N9-ethyl substitution is optimum for inhibitory activity against pcDHFR and tgDHFR for the 2,4-diaminoquinazolines. Selectivity was unaffected by N9 substitution.

Introduction

Opportunistic infections, such as *Pneumocystis pneumonia* (PCP^a) and toxoplasmosis, remain a major cause of mortality in acquired immunodeficiency syndrome (AIDS) patients.^{1–3} Trimethoprim (TMP) (Figure 1) is a relatively weak inhibitor ($K_i = 25 \pm 7$ nM) of dihydrofolate reductase (DHFR) from *P. jirovecii*, the form of *Pneumocystis* found in humans; TMP is even less effective ($K_i = 770 \pm 340$ nM) against the DHFR found in *P. carinii* (pc), the organism that infects rats. In humans and in the drug testing model in rats, TMP must be coadministered with sulfonamides to provide synergistic antifolate effects.^{4,5} Similarly, when pyrimethamine, a rather nonselective DHFR inhibitor, is used to treat toxoplasmosis, it is necessary to combine the weak DHFR inhibitor with a sulfonamide to achieve synergistic effects. In both of these situations, the side effects associated with sulfonamides often result in discontinuation of therapy.⁶

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Supporting Information Available: Detailed elemental analysis data for compounds **5–13**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

^aAbbreviations: pc, *Pneumocystis carinii*; tg, *Toxoplasma gondii*; DHFR, dihydrofolate reductase; HIV, human immunodeficiency virus; PCP, *Pneumocystis pneumonia*; AIDS, acquired immunodeficiency syndrome; TMP, Trimethoprim; PTX, piritrexim; TMQ, trimetrexate; ma, *Mycobacterium avium*.

Lipophilic, nonclassical antifolates, such as trimetrexate (TMQ) (Figure 1), can passively diffuse into the pathogenic cells, as well as host cells. TMQ is 286-fold and 159-fold more potent against pcDHFR and tgDHFR, respectively, than TMP, but TMQ is not selective for the mammalian DHFR and thus must be coadministered with the classical folate leucovorin (5-formyltetrahydrofolate) due to lack of selectivity.^{7–9} Host tissues can be selectively rescued because leucovorin is only taken up by the mammalian transport system(s). Drawbacks of the TMQ-leucovorin regimen are the high cost and variable effectiveness of leucovorin.¹⁰ Thus, the search for single agents that are potent and selective against pcDHFR and/or tgDHFR continues in several laboratories.

Gangjee et al.,^{11–16} as well as others,^{17–21} have reported several structural classes of DHFR inhibitors. Selectivity is reported as the ratio of the IC₅₀ value of mammalian DHFR/IC₅₀ value of pcDHFR or tgDHFR. A series of 2,4-diaminopyrido[2,3-*d*]pyrimidines were reported by Gangjee et al.⁹ as two atom-bridged variations of the potent nonclassical antifolate piritrexim (PTX).²² Compound **1** (see Figure 2) showed moderate DHFR inhibitory potency with IC₅₀ values of 3.80 and 0.31 μM against pcDHFR and tgDHFR, respectively. N9-methylation of **1** to afford **2** resulted in a 45-fold and 49-fold increase in potency against pcDHFR (IC₅₀ = 84 nM) and tgDHFR (IC₅₀ = 6.3 nM), respectively. The selectivity ratio for tgDHFR versus recombinant human (rh) DHFR of compound **2** was 304. Thus, N9-methylation in the reverse-bridged analogue significantly increased potency against pcDHFR and tgDHFR, as well as selectivity for tgDHFR versus rhDHFR. However, **2** was 447-times less potent against *T. gondii* cells in culture compared to isolated tgDHFR. These results suggest inadequate cell penetration.

To explore the effect on potency, selectivity, and cell penetration, Gangjee et al.²³ synthesized a series of analogues of compound **2** with an isosteric replacement of the N8 with a carbon to yield 2,4-diaminoquinazolines with increased lipophilicity. A comparison of the potency and selectivity of the quinazoline analogues with the pyrido[2,3-*d*]pyrimidines with identical side chain substituents revealed different structure–activity/selectivity relationships for the two classes. Contrary to the pyrido[2,3-*d*]pyrimidines, in most cases, the N9—H quinazoline analogues showed better selectivity for tgDHFR versus rhDHFR and tgDHFR versus hDHFR than their corresponding N9-methyl analogues. The N9-methyl quinazolines were, however, considerably more potent against both pcDHFR and tgDHFR than the N9—H quinazolines. Three trimethoxyphenyl substituted N9—H quinazolines were selected for evaluation in *T. gondii* cell culture studies. The cell culture/enzyme ratio (IC₅₀ value in *T. gondii* cell culture/IC₅₀ value in tgDHFR) ranged from 3 to 142, which was significantly better than the ratio of 447 for compound **2**. This increase in inhibition of *T. gondii* cells in culture was attributed, in part, to the increased lipophilicity of the quinazolines versus the pyrido-[2,3-*d*]pyrimidines and provided the impetus to explore further modifications of quinazolines to improve selectivity and potency.²³

X-ray crystal structures of 5-deaza-5-methyl antifolates show a hydrophobic interaction between the 5-methyl moiety and Val115 in hDHFR.²⁴ In the crystal structure of PTX complexed with pcDHFR, a hydrophobic interaction between the 5-methyl moiety and Ile123 is observed.²⁵ The fact that the 5-methyl moiety of PTX has hydrophobic interactions in both pcDHFR and hDHFR may explain, in part, the lack of selectivity of PTX. The X-ray crystal structures of **2** complexed with hDHFR²³ and pcDHFR²⁶ showed that the distance between the N9-methyl moiety of **2** and the Val115 (hDHFR) or Ile123 (pcDHFR)²⁵ was 4.66 Å and 4.20 Å, respectively,²⁷ beyond the range for van der Waals interaction (4.00 Å). The Val115 of hDHFR corresponds to Ile123 in pcDHFR. Because the quinazolines demonstrated better *T. gondii* cell to tgDHFR inhibitory ratios and the N9-methyl moiety of the pyrido[2,3-*d*]pyrimidines did not interact with Ile123, it was of interest to increase the

size of the N9-alkyl substitution in the quinazoline series. It was anticipated that this increase would provide both potent and selective inhibitors.

Thus, the N9-alkyl 2,4-diaminoquinazolines **5–13** were designed and synthesized as analogues of compound **4**. Compound **5** was synthesized as a phenyl ring unsubstituted analogue. Compounds **6** and **7** contain α - and β -naphthyl moieties. A naphthyl group has been shown by Gangjee et al.²⁸ to interact with Phe69 in pcDHFR. Compound **9** contains 2,3,5-trichloro substituents to determine the effect of electron withdrawing groups on biological activity. The N9-propyl analogues **10** and **11** and the cyclopropyl methyl analogues **12** and **13** were synthesized to determine the importance of the size of the N9-alkyl moiety on biological activity. The increase in the number of carbons at the N9-position contributes to the lipophilicity of the analogues and also serves as a site for possible hydrophobic interactions with Val115 of hDHFR, Ile123 of pcDHFR, and/or hydrophobic residues in tgDHFR. The N9-ethyl substituted compounds **5–9** were designed to determine whether potency and/or selectivity can be increased by decreasing the distance between the N9-alkyl moiety and the hydrophobic residues in the DHFR binding site. The N9-propyl and -cyclopropyl methyl analogues **10–13** were designed to determine whether the increase in the number of carbons from 2 to 3 or 4 would increase the potential hydrophobic interactions or exceed the optimum distance between the DHFR residues and the N9-moiety.

Chemistry

The synthesis of target compounds **5–13** began with commercially available 2,4-diamino-6-nitroquinazoline **14** and is outlined in Scheme 1.²³ Starting material **14** was reduced with hydrogen at 30–35 psi and Raney nickel catalyst for 3 h to afford the key intermediate 2,4,6-triaminoquinazoline **15**. To the reaction vessel was added the appropriate benzaldehyde or naphthaldehyde, and the reaction mixture was hydrogenated for an additional 15 h to afford **16–20**, the N9—H precursors to the target compounds, in yields ranging from 36% to 82%, depending on the phenyl substitution. Reductive N9-alkylation with acetaldehyde, propionaldehyde, or cyclopropanecarboxaldehyde using sodium cyanoborohydride afforded the target compounds **5–13** in yields ranging from 9% to 67%, depending on the aldehyde and the phenyl substitution.

Biological Activity and Discussion

Compounds **5–13** were evaluated as inhibitors of pcDHFR, tgDHFR, rDHFR, and *Mycobacterium avium* (ma) DHFR, and the results are reported in Table 1. In general, the N9-ethyl substituted analogues were more potent than the unsubstituted or N9-methyl substituted compounds against pcDHFR and, in most cases, against tgDHFR as well. Against pcDHFR, the α -naphthyl compound **6** was the most potent. Against tgDHFR, the unsubstituted phenyl analogue **5** and the 2,5-diOCH₃ phenyl analogue **8** were most potent. The α -naphthyl analogue **6** and the β -naphthyl analogue **7** were 8-fold and 3-fold more potent against pcDHFR, respectively, than the unsubstituted phenyl analogue **5**. This suggests a tolerance for larger side chain rings in the pcDHFR binding site. Against tgDHFR, compounds **6** and **7** were 2-fold and 3-fold less potent, respectively, than **5**, which suggests the naphthyl rings bind less favorably than the phenyl ring, contrary to that observed for pcDHFR. Substitution of electron-donating methoxy groups on the side chain phenyl ring in **8** increased potency against pcDHFR relative to **5**. Against tgDHFR, **5** and **8** were equipotent. Electron-withdrawing chloro groups on the phenyl ring also resulted in an increase in potency against pcDHFR for compound **9**. The fact that methoxy and chloro groups both contributed to increased potency against pcDHFR compared to the unsubstituted phenyl ring suggests that the increase was not due to electronic factors but was more likely due to favorable binding conformations induced by the substitutions on the phenyl ring in compounds **8** and **9** compared to **5**. Against tgDHFR, electron-withdrawing

chloro substituents on the side chain phenyl ring results in a 5-fold decrease in inhibitory potency compared to the unsubstituted phenyl ring, with IC₅₀ values for compounds **5** and **9** of 3.6 and 19 nM, respectively.

The effect on inhibitory potency against pcDHFR and tgDHFR of increasing the number of carbons at the N9-position in the quinazoline series can be deduced from a comparison of IC₅₀ values for the 2,5-dimethoxy phenyl substituted analogues **3**, **4**, **8**, **10**, and **12**. As previously reported,²³ the potency against pcDHFR increased 53-fold upon N9-methylation, with IC₅₀ values of 4600 and 87 nM for **3** and **4**, respectively. N9-substitution with the homologous ethyl group to yield compound **8** resulted in a 9-fold increase in potency against pcDHFR compared to the N9-methyl analogue **4**, with an IC₅₀ value of 9.9 nM for **8**. However, propyl substitution at the N9-position caused the inhibitory potency against pcDHFR to decrease. The N9-propyl analogue **10** showed an IC₅₀ value of 38 nM, 4-fold less than **8**. The N9-cyclopropyl methyl analogue **12** had a slight increase in potency against pcDHFR compared to **10**, with an IC₅₀ value of 25 nM. Despite the N9-cyclopropyl methyl group having one additional carbon compared to the N9-propyl moiety, the similarity in IC₅₀ values for **10** and **12** was consistent with the similarity in carbon chain lengths in the two groups. The increase in inhibitory potency against pcDHFR upon N9-ethylation is also shown in the α -naphthyl quinazoline analogues. The IC₅₀ values for the previously reported N9-H and N9-methyl α -naphthyl quinazolines²³ were 720 and 17 nM, respectively, indicating a 42-fold increase in potency against pcDHFR upon N9-methylation. The N9-ethyl analogue **6** showed an IC₅₀ value of 2.5 nM and was 7-fold more potent against pcDHFR than the N9-methyl analogue. Also, as seen in the 2,5-dimethoxy series, in the β -naphthyl quinazoline analogues, replacement of the N9-ethyl group with a propyl or cyclopropyl methyl group resulted in a decrease in potency against pcDHFR. The N9-ethyl analogue **7** was 4-fold and 6-fold more potent than **11** and **13**, respectively.

Against tgDHFR, compound **4** was 5-fold more potent than **3**, illustrating that just as for pcDHFR, N9-ethylation resulted in an increase in potency against tgDHFR compared to the N9-methyl analogue. Compound **8** was 8-fold more potent than **4** against tgDHFR. However, the N9-propyl homologue **10** showed a 3-fold decrease in potency compared to **8**. The N9-cyclopropyl methyl analogue **12** showed a very similar potency against tgDHFR compared to **10**. Potency against tgDHFR also increased with N9-ethylation in the α -naphthyl quinazoline analogues. The N9-ethyl analogue **6** showed a 3-fold increase in potency compared to the N9-methyl analogue against tgDHFR. In the β -naphthyl quinazoline analogues **7**, **11**, and **13** reported herein, the increase in the number of carbons at the N9-position caused about a 2-fold reduction in potency against tgDHFR.

Selectivity for pcDHFR in the quinazoline analogues is generally low. N9-substituents with increasing numbers of carbons did not significantly improve selectivity for pcDHFR. Compounds **3** and **4** both showed increased potency against rIDHFR compared to pcDHFR with selectivity ratios of 0.24 and 0.29, respectively. N9-ethyl, -propyl, or -cyclopropyl methyl substitutions (compounds **8**, **10**, and **12**), in general, did not improve selectivity for pcDHFR. Only compounds **6**, **7**, and **9** showed greater potency against pcDHFR compared to rIDHFR with marginal increase in selectivity ratios. N9-propylation and N9-cyclopropylmethylation of the β -naphthyl quinazoline resulted in a decrease in selectivity for pcDHFR compared to **7**.

Selectivity for tgDHFR in the N9-H quinazoline analogues was generally higher than selectivity for pcDHFR, with selectivity ratios of 7 and 0.2 for tgDHFR and pcDHFR, respectively, for the 2,5-dimethoxyphenyl analogue **3**. N9-alkylation resulted in a decrease in selectivity for tgDHFR; compounds **8**, **10**, and **12** showed selectivity ratios of 1.3, 0.6, and 1.3, respectively, for tgDHFR. The selectivity for tgDHFR of the N9-ethyl, -propyl, and

-cyclopropyl analogues was low, indicating a minimal but consistent decrease in selectivity for tgDHFR with a number of carbons greater than one in the N9-position.

Compounds **5–13** were also evaluated as inhibitors of maDHFR. The N9-ethyl dimethoxyphenyl analogue **8** was the most potent with an IC₅₀ value of 0.98 nM. Compound **8** was equipotent compared to the unsubstituted phenyl analogue **5**. The α - and β -naphthyl analogues **6** and **7** were slightly less potent. Compound **9**, with the electron-withdrawing 2,3,5-trichloro substituents on the side chain phenyl ring, was 13-fold less potent than **5** against maDHFR. N9-propylation of the 2,5-dimethoxy quinazoline resulted in a 3-fold decrease in potency for **10** against maDHFR compared to **8**. Cyclopropyl methylation at the N9-position decreased potency against maDHFR by 5-fold for **12** compared to **10**. N9-propylation of the β -naphthyl quinazoline resulted in a 6-fold decrease in potency against maDHFR for **11** compared to **7**. The N9-cyclopropyl methyl β -naphthyl quinazoline **13** was 3-fold less potent compared to **11**.

The unsubstituted phenyl analogue **5** was selective for maDHFR versus rIDHFR with a selectivity ratio of 3.7. The α -naphthyl analogue **6** was less selective compared to **5** with a selectivity ratio of 2.2. However, the β -naphthyl analogue **7** was more selective for maDHFR compared to **5** with a selectivity ratio of 6.3 and was the most selective analogue for maDHFR reported herein. Compound **8**, with electron-donating 2,5-dimethoxy substituents on the phenyl ring, was more selective for maDHFR compared to **5**. The decrease in selectivity for maDHFR of **9** (selectivity ratio = 1.4), with electron-withdrawing 2,3,5-trichloro substituents on the phenyl ring, suggests that selectivity for maDHFR versus rIDHFR may be affected by the electronic nature of the phenyl ring substitutions. Selectivity for maDHFR was generally greater for the N9-ethyl quinazoline analogues and decreased with an increase in the number of carbons at the N9-position. N9-propyl analogues **10** (selectivity ratio = 1.6) and **11** (selectivity ratio = 1.3) were 3-fold and 5-fold less selective compared to **8** and **7**, respectively. N9-cyclopropylmethylation resulted in analogues that were more selective for rIDHFR versus maDHFR with selectivity ratios for **12** and **13** of less than one.

Experimental Section

All evaporations were carried out in vacuo with a rotary evaporator. Analytical samples were dried in vacuo over P₂O₅. Thin-layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator. Spots were visualized by UV light (254 and 365 nm). All analytical samples were homogeneous on TLC in at least two different solvent systems. Purification by column chromatography was carried out using Merck silica gel 60 (200–400 mesh). The amount of silica gel used for column chromatography was depended upon the chemical nature and amount of the compounds being separated. Columns were usually wet-packed unless otherwise noted. Solvent systems are reported as volume percent mixtures. Melting points were determined on a Mel-Temp II melting point apparatus with a digital thermometer and are uncorrected. ¹H NMR spectra were recorded on a Bruker WH-300 (300 MHz) NMR spectrometer. The chemical shift (δ) values are reported as parts per million (ppm) relative to tetramethylsilane as internal standard: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad peak, exch = exchangeable protons by addition of D₂O. Elemental analyses were performed by Atlantic Microlabs, Inc., Norcross, GA. Elemental compositions were within $\pm 0.4\%$ of the calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples of antifolates could not be removed despite 24 h of drying in vacuo and were confirmed, where possible, by their presence in the ¹H NMR spectrum. All solvents and chemicals were purchased from Aldrich Chemical Co. and Fisher Scientific Co. and were used as received except where anhydrous solvents were needed, which were freshly dried in the laboratory.

General Procedure for the Synthesis of Compounds 5–13

To the 2,4-diamino[*N*-substituted benzyl]amino]quinazoline suspended in CH₃CN was added acetaldehyde, propionaldehyde, or cyclopropanecarboxaldehyde, followed by NaCNBH₃. The suspension was pH adjusted to 2–3 by dropwise addition of concentrated HCl. As the mixture was acidified, the suspended starting material began to dissolve into solution, followed by precipitation of the crude product. In some cases, dissolution was not seen but a visible color change occurred. The suspension containing the crude product was stirred for an additional 0.5 h. The crude product was filtered, stirred in 2 N Na₂CO₃, and washed with H₂O, C₂H₅OH, and C₂H₅OC₂H₅. The purity of each product was checked by TLC using a solvent system of 5:2:0.1 (A) or 5:1:0.1 (B) by volume of CHCl₃:CH₃OH:NH₄OH. Crude products were dissolved in CH₃OH, pH adjusted to 8 with NH₄OH, added to silica gel, followed by the evaporation of the solvent to afford a silica gel plug. The dry plug containing the product was placed on a column of wet silica gel and eluted with solvent B or a solvent system of 10:1 (C) by volume of CHCl₃:CH₃OH. Pure fractions, identified as single spots by TLC, were collected and the solvent evaporated to yield analytically pure solids.

2,4-Diamino-6-[*N*-(benzyl)-*N*-ethylamino]quinazoline 5

Compound **5** was synthesized from **16** (0.20 g, 0.75 mmol), acetaldehyde (0.20 mL, 3.6 mmol), and NaCNBH₃ (0.125 g, 2.0 mmol) in 50 mL of CH₃CN. Compound **5** was filtered from the reaction mixture to yield an analytically pure bright-yellow solid (0.10 g, 45%): mp 280–282 °C; TLC (solvent A) *R_f* 0.46. ¹H NMR (DMSO-*d*₆) δ 1.11 (t, 3 H, N-CH₂-CH₃), 3.48 (q, 2 H, N-CH₂-CH₃), 4.60 (s, 2 H, 10-CH₂), 7.24–7.38 (m, 10 H, Ar, NH₂), 8.6 (br, 2 H, NH₂). Anal. (C₁₇H₁₉N₅ • 1.8HCl • 0.6CH₃OH) C, H, N.

2,4-Diamino-6-[*N*-(α-naphthyl)-*N*-ethylamino]quinazoline 6

Compound **6** was synthesized from **17** (0.47 g, 1.50 mmol), acetaldehyde (0.40 mL, 7.2 mmol), and NaCNBH₃ (0.25 g, 4.0 mmol) in 50 mL of CH₃CN. Compound **6** was filtered from the reaction mixture to yield an analytically pure solid (0.20 g, 39%): mp 266–267 °C; TLC (solvent B) *R_f* 0.40. ¹H NMR (DMSO-*d*₆) δ 1.16 (t, 3 H, N-CH₂-CH₃), 3.49–3.56 (q, 2 H, N-CH₂-CH₃), 5.05 (s, 2 H, 10-CH₂), 7.18–7.21 (m, 2 H, Ar), 7.27–7.30 (m, 1 H, Ar), 7.39–7.62 (m, 4 H, Ar, 2 H, NH₂), 7.82–7.85 (d, 1 H, Ar), 7.96–7.99 (m, 1 H, Ar), 8.08–8.11 (m, 1 H, Ar), 8.60 (s, 2 H, NH₂), 8.92 (s, 2 H, NH₂). Anal. (C₂₁H₂₁N₅ • 1.5HCl) C, H, N.

2,4-Diamino-6-[*N*-(β-naphthyl)-*N*-ethylamino]quinazoline 7

Compound **7** was synthesized from **18** (0.32 g, 1.01 mmol), acetaldehyde (0.27 mL, 4.85 mmol), and NaCNBH₃ (0.17 g, 2.7 mmol) in 50 mL of CH₃CN. Compound **7** was filtered from the reaction mixture to yield an analytically pure solid (0.23 g, 67%): mp 278–280 °C; TLC (solvent B) *R_f* 0.45. ¹H NMR (DMSO-*d*₆) δ 1.12–1.17 (t, 3 H, N-CH₂-CH₃), 3.56–3.58 (q, 2 H, N-CH₂-CH₃), 4.77 (s, 2 H, 10-CH₂), 7.27 (s, 2 H, Ar), 7.40–7.50 (m, 4 H, Ar, 2 H, NH₂), 7.72 (s, 1 H, Ar), 7.81–7.89 (m, 3 H, Ar), 8.62 (br, 2 H, NH₂). Anal. (C₂₁H₂₁N₅ • 1.0HCl) C, H, N.

2,4-Diamino-6-[*N*-(2,5-dimethoxybenzyl)-*N*-ethylamino]quinazoline 8

Compound **8** was synthesized from **19** (0.24 g, 0.75 mmol), acetaldehyde (0.20 mL, 3.6 mmol), and NaCNBH₃ (0.125 g, 2.0 mmol) in 50 mL of CH₃CN. Compound **8** was purified by column chromatography to yield an analytically pure bright-yellow solid (0.16 g, 59%): mp 200–204 °C; TLC (solvent B) *R_f* 0.43. ¹H NMR (DMSO-*d*₆) δ 1.05 (br, 3 H, N-CH₂-CH₃), 3.36–3.38 (br, 2 H, N-CH₂-CH₃), 3.52 (s, 3 H, OCH₃), 3.73 (s, 3 H, OCH₃), 4.38 (s, 2 H, 10-CH₂), 5.66 (br, 2 H, NH₂), 6.52 (s, 1 H, Ar), 6.72 (m, 1 H, Ar), 6.86–6.94 (m, 3 H,

Ar), 7.02–7.05 (d, 1 H, Ar), 7.12–7.17 (br, 2 H, NH₂). Anal. (C₁₉H₂₃N₅O₂ • 0.7H₂O) C, H, N.

2,4-Diamino-6-[*N*-(2,3,5-trichlorobenzyl)-*N*-ethylamino]quinazoline 9

Compound **9** was synthesized from **20** (0.20 g, 0.55 mmol), acetaldehyde (0.15 mL, 2.7 mmol), and NaCNBH₃ (0.10 g, 1.65 mmol) in 20 mL of CH₃CN. Compound **9** was purified by column chromatography to yield an analytically pure bright-yellow solid (0.05 g, 21%): mp 222–224 °C; TLC (solvent B) R_f 0.46. ¹H NMR (DMSO-*d*₆) δ 1.1–1.14 (t, 3 H, N-CH₂-CH₃), 3.45–3.49 (q, 2 H, N-CH₂-CH₃), 4.56 (s, 2 H, 10-CH₂), 5.65 (s, 2 H, NH₂), 7.05–7.06 (d, 2 H, Ar), 7.11–7.16 (m, 4 H, Ar, NH₂), 7.74–7.75 (d, 1 H, Ar). Anal. (C₂₁H₂₁N₅Cl₃ • 0.3H₂O) C, H, N, Cl.

2,4-Diamino-6-[*N*-(2,5-dimethoxybenzyl)-*N*-propylamino]quinazoline 10

Compound **10** was synthesized from **19** (0.20 g, 0.62 mmol), propionaldehyde (0.11 g, 1.8 mmol), and NaCNBH₃ (0.116 g, 1.8 mmol) in 50 mL of CH₃CN. Compound **10** was purified by column chromatography to yield an analytically pure solid (13%): mp 172–173 °C; TLC (solvent B) R_f 0.58. ¹H NMR (DMSO-*d*₆) δ 0.86–0.91 (t, 3 H, N-CH₂-CH₂-CH₃), 1.53–1.58 (m, 2 H, N-CH₂-CH₂-CH₃), 3.31 (br, N-CH₂-CH₂-CH₃), 3.56 (s, 3 H, OCH₃), 3.78 (3.3 H, OCH₃), 4.46 (s, 2 H, 10-CH₂), 5.71 (s, 2 H, NH₂), 6.52–6.53 (d, 1 H, Ar), 6.72–6.76 (m, 1 H, Ar), 6.90–7.00 (m, 2 H, Ar), 7.07–7.14 (d, 2 H, Ar), 7.23 (br, 2 H, NH₂). Anal. (C₂₀H₂₅N₅O₂ • 0.5H₂O) C, H, N.

2,4-Diamino-6-[*N*-(β-naphthyl)-*N*-propylamino]quinazoline 11

Compound **11** was synthesized from **18** (0.20 g, 0.63 mmol), propionaldehyde (0.74 g, 12.7 mmol), and NaCNBH₃ (0.12 g, 2.0 mmol) in 50 mL of CH₃CN. Compound **11** was purified by column chromatography to yield an analytically pure solid (46%): mp 189.0–190.7 °C; TLC (solvent B) R_f 0.42. ¹H NMR (DMSO-*d*₆) δ 0.86–0.91 (t, 3 H, N-CH₂-CH₂-CH₃), 1.56–1.66 (t, 2 H, N-CH₂-CH₂-CH₃), 4.71 (s, 2 H, 10-CH₂), 5.67 (br, 2 H, NH₂), 7.21–7.29 (m, 4 H, Ar, NH₂), 7.40–7.46 (m, 3 H, Ar), 7.72 (s, 1 H, Ar), 7.79–7.86 (m, 3 H, Ar). Anal. (C₂₂H₂₃N₅ • 0.3H₂O) C, H, N.

2,4-Diamino-6-[*N*-(2,5-dimethoxybenzyl)-*N*-cyclopropyl methylamino]quinazoline 12

Compound **12** was synthesized from **19** (0.20 g, 0.62 mmol), cyclopropanecarboxaldehyde (0.86, 12.3 mmol), and NaCNBH₃ (0.12 g, 1.8 mmol) in 50 mL of CH₃CN. Compound **12** was purified by column chromatography to yield an analytically pure solid (21%): mp 122.7–123.5 °C; TLC (solvent B) R_f 0.54. ¹H NMR (DMSO-*d*₆) δ 0.18–0.19 (d, 2 H, N-CH₂-cyclopropyl), 0.41–0.44 (d, 2 H, N-CH₂-cyclopropyl), 1.06–1.08 (m, 1 H, N-CH₂-cyclopropyl), 3.29–3.31 (d, 2 H, N-CH₂-cyclopropyl), 3.56 (s, 3 H, OCH₃), 3.79 (3.3 H, OCH₃), 4.56 (s, 2 H, 10-CH₂), 5.80 (br, 1 H, NH₂), 6.58 (s, 1 H, Ar), 6.72–6.75 (d, 1 H, Ar), 6.90–6.93 (d, 1 H, Ar), 7.0–7.035 (d, 1 H, Ar), 7.08–7.10 (d, 1 H, Ar), 7.24 (s, 1 H, Ar), 7.29 (br, 1 H, NH₂). Anal. (C₂₁H₂₅N₅O₂ • 0.6H₂O) C, H, N.

2,4-Diamino-6-[*N*-(β-naphthyl)-*N*-cyclopropyl methylamino]quinazoline 13

Compound **13** was synthesized from **18** (0.20 g, 0.63 mmol), cyclopropanecarboxaldehyde (0.89 g, 12.7 mmol), and NaCNBH₃ (0.12 g, 2.0 mmol) in 50 mL of CH₃CN. Compound **13** was purified by column chromatography to yield an analytically pure solid (9%): mp 136–138 °C (dec); TLC (solvent B) R_f 0.58. ¹H NMR (DMSO-*d*₆) δ 0.20–0.21 (d, 2 H, N-CH₂-cyclopropyl), 0.42–0.45 (d, 2 H, N-CH₂-cyclopropyl), 1.06–1.11 (m, 1 H, N-CH₂-cyclopropyl), 4.80 (s, 2 H, 10-CH₂), 6.00 (br, 1 H, NH₂), 7.08 (d, 1 H, Ar), 7.21 (d, 1 H, Ar), 7.32 (s, 1 H, Ar), 7.43 (m, 4 H, Ar), 7.24 (s, 1 H, Ar), 7.74–8.00 (m, 3 H, Ar, NH₂). Anal. (C₂₃H₂₃N₅ • 1.1H₂O) C, H, N.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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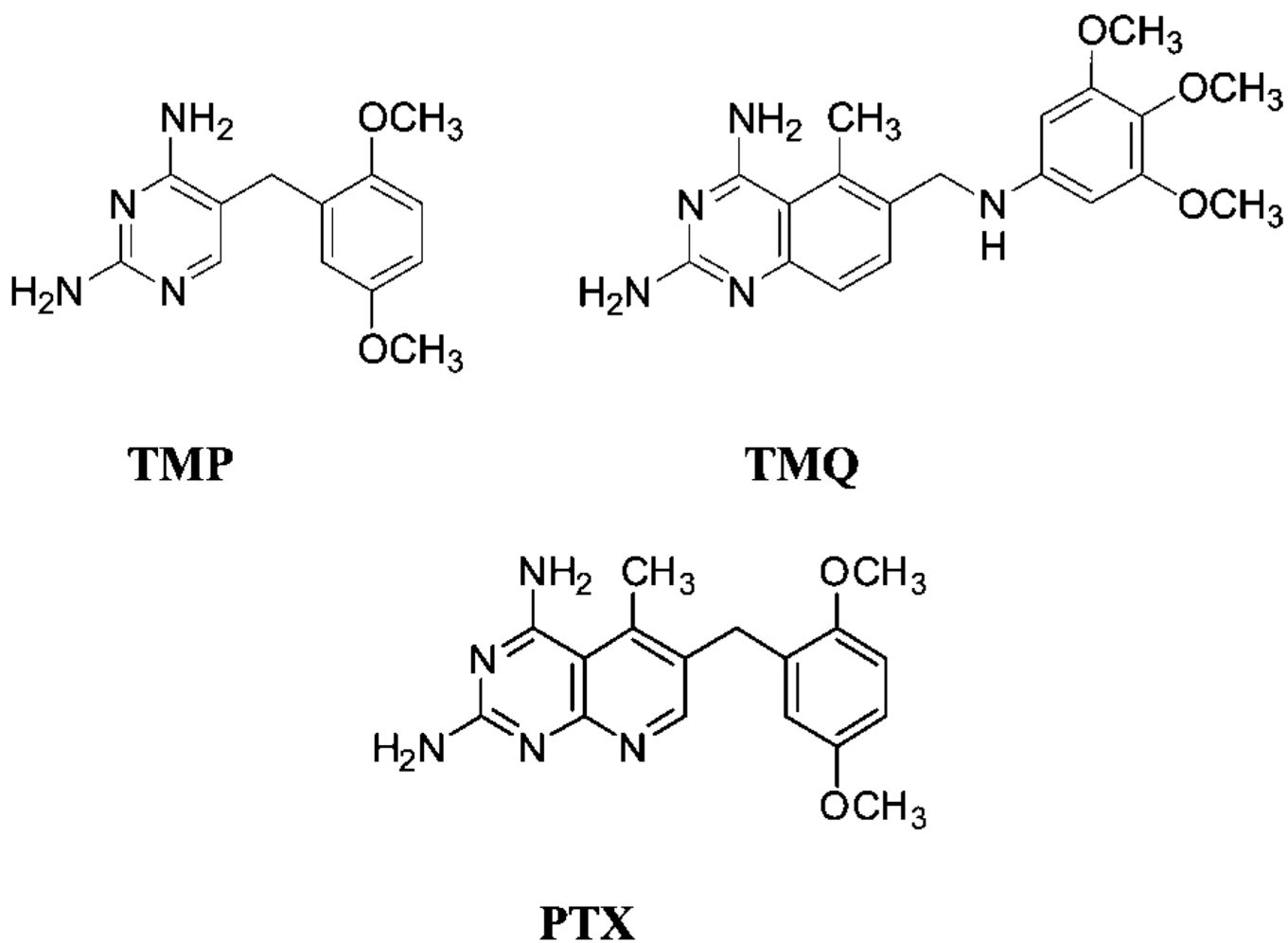
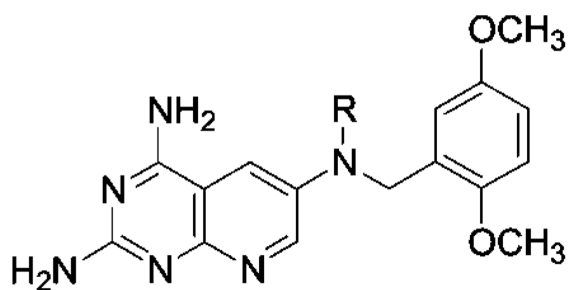
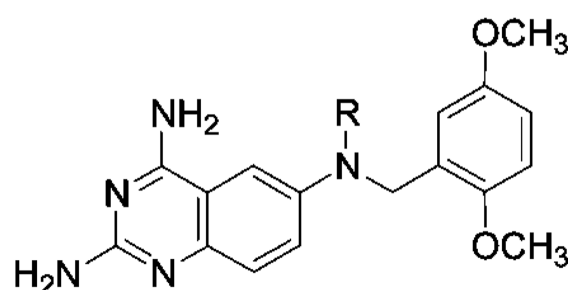


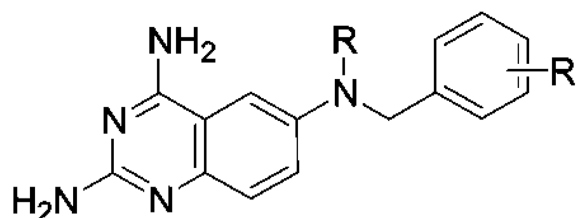
Figure 1.



1. $R = H$
2. $R = CH_3$

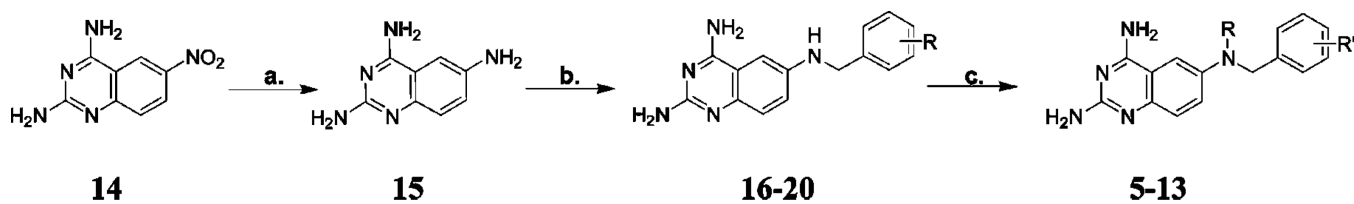


3. $R = H$
4. $R = CH_3$



- | <u>R</u> | <u>R'</u> |
|--------------|------------------|
| 5. C_2H_5 | H |
| 6. C_2H_5 | $2',3'-C_4H_4$ |
| 7. C_2H_5 | $3',4'-C_4H_4$ |
| 8. C_2H_5 | $2',5'-diOCH_3$ |
| 9. C_2H_5 | $2',3',5'-triCl$ |
| 10. C_3H_7 | $2',5'-diOCH_3$ |
| 11. C_3H_7 | $3',4'-C_4H_4$ |
| 12. C_4H_7 | $2',5'-diOCH_3$ |
| 13. C_4H_7 | $3',4'-C_4H_4$ |

Figure 2.

**Scheme 1^a**

Reagents: (a.) Raney nickel, DMF, H₂ at 30–35 psi; (b.) Raney nickel, DMF, CH₃COOH, ArCHO, H₂ at 30–35 psi; (c.) RCHO, NaCNBH₃, HCl, CH₃CN.

Inhibition Concentrations (IC_{50} , in nM) against DHFR from *P. carinii* (pc), *T. gondii* (tg), *M. avium* (ma), and Rat Liver (rl) and Selectivity Ratios^a

Table 1

R	R'	pcDHFR	riDHFR	ri/pc	tgDHFR	ri/tg	maDHFR	rl/ma
1	H	3800	350	0.09	310	1.1		
2	CH ₃	84	57	0.7	6.3	9.0		
3	H	4600	1100	0.24	160	6.88		
4	CH ₃	87	26	0.29	30	0.86		
5	C ₂ H ₅	21	4.1	0.2	3.6	1.1	1.1	3.73
6	C ₂ H ₅	2.5	6.5	2.6	7.8	0.8	2.9	2.24
7	C ₂ H ₅	7.2	14.5	2.0	12	1.2	2.3	6.30
8	C ₂ H ₅	9.9	4.8	0.5	3.7	1.3	0.98	4.90
9	C ₂ H ₅	6.9	19	2.8	19	1.0	14	1.36
10	C ₃ H ₇	38	5.3	0.1	9.5	0.6	3.3	1.63
11	C ₄ H ₇	27	17	0.6	19	0.9	13	1.31
12	C ₄ H ₇	25	9.6	0.4	7.3	1.3	16	0.60
13	C ₃ H ₇	42	16	0.4	25	0.6	34	0.47
TMP ^b		12000	133000	11.1	2,700	49		
TMQ ^b		42	3.0	0.07	10	0.3		

^aThese assays were carried out at 37 °C under saturating conditions of substrate (0.092 mM dihydrofolic acid) and cofactor (0.117 mM NADPH); riDHFR and tgDHFR were assayed in the presence of 150 mM KCl.

^bThese values are from ref 28. Most recent quality control values for riDHFR are 121400 ± 7500 nM for TMP ($R^2 = 0.9768$), 8.0 ± 0.5 nM ($R^2 = 0.9561$) for TMQ and 4.4 ± 0.4 nM ($R^2 = 0.9386$) for PTX. Most recent quality control values for pcDHFR are 26,820 ± 1700 nM ($R^2 = 0.995$) for TMP, 47 ± 13 nM ($R^2 = 0.923$) for TMQ, and 34.3 ± 10 nM ($R^2 = 0.9876$) for PTX. These quality control assays used recombinant pcDHFR, as did all the assays for experimental compounds shown above. Published data on TMP, TMQ and PTX used native pcDHFR; side by side comparisons show no difference in drug susceptibility between the two forms of pcDHFR.